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THE DNA SEQUENCE ENCODING GLYCERALDEHYDE 3-PHOSPHATE DEHYDROGENASE (GAPC) ENZYME ON TUNTUN ANGIN PLANT (ELAEOCARPUS FLORIBUNDUS BI)

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Abstract

Glyceraldehyde 3-phosphate dehydrogenase (GapC) is an enzyme involved in glycolysis. The expression of this gene tends to abundant in eukaryotic cells, so this gene is frequently used as an internal control in gene expression analysis. This research aims to isolate the DNA sequence of the GapC gene from tuntun angin (Elaeocarpus floribundus BI). Methods included a collection of the leaves from Kajuik Lake, Riau Province then the DNA extraction, electrophoresis, amplification of partial DNA sequence of GapC gene, cloning and sequencing. The DNA sequence was analyzed using the BLASTn program and MEGA6 software. The GapC sequence obtained in this study was 933 bp in size, consisting of four introns and five exons, and encoding 137 deduced amino acids. The BLASTn analysis showed that the sequence has 89.84%-90.16% similarity to other species of Cunoniaceae family, such as species from the genus of Spiraeanthemum and Codia. The parsial sequence of E. floribundus GapC gene was more resemble the one of Spiraeanthemum than Codia genus. The GapC sequence obtained in this study was the first reported from the Elaeocarpaceae family. This sequence has the opportunity to serve as an internal control after validation.

Keywords: *GapC gene, Elaeocarpus floribundus, internal control, Riau.*

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INTRODUCTION

The *Elaeocarpus floribundus* BI plant is a species of the *Elaeocarpaceae* family that grows in Lake Kajuik, Riau Province (Elvyra & Yus, 2012; Roslim et al., 2016). This plant has adapted well to the flood exposure ecosystem, that is, this plant can grow well in flooded conditions for months during the rainy season. This shows that this plant is most likely to carry inundation-tolerant genes.

To study the theory that underlies the tolerance of plants to inundation stress, it is necessary to analyze the expression of these genes. For this purpose several reference genes are needed which act as internal controls in gene expression studies (Ezin et al., 2012; Wang et al., 2017). One gene that is often used as internal control in plants is the gene coding for the enzyme glyceraldehyde 3-phosphate dehydrogenase (*GapC*) (Kozera & Rapacz, 2013). In wheat plants (*Triticum aestivum*) 4 groups of gene coding for the enzyme glyceraldehyde 3-phosphate dehydrogenase (GAPDH) were identified, namely *GapA / B, GapC, GapCp, and GapN* (Zeng et al., 2016).

This gene is involved in *glycolysis* and is expressed in abundant amounts in plant cells. The universal primer for amplifying this gene has been designed by *Strand* et al. (1997).

Therefore, this study aims to isolate partial DNA sequences of the *GapC* gene in *E. floribundus* plants.

MATERIALS AND METHODS

The research was conducted from January to April 2019. The research material was in the form of leaves from *Elaeocarpus floribundus* taken from Kajuik Lake in Riau Province. Molecular analysis was carried out in the Genetics laboratory, Department of Biology, FMIPA, Riau University, Indonesia. The primers used are as follows: GPDX7F: 5'-GAT AGA TTT GGA ATT GTT GAG G-3 'and GPDX9R: 5'-AAG CAA TTC CAG CCT TGG-3' (Strand et al., 1997; Wu et al. , 2007).

Total of DNA Isolation. The total DNA molecule from the *E. floribundus* plant was isolated using the Genomic DNA Mini Kit (Plant, Geneaid GP100). 100 mg of leaves were crushed using mortar and pestle and assisted with liquid nitrogen. The total DNA molecule obtained was then migrated to 1.2% agarose gel in a 1X TBE buffer solution to determine the success of DNA isolation.

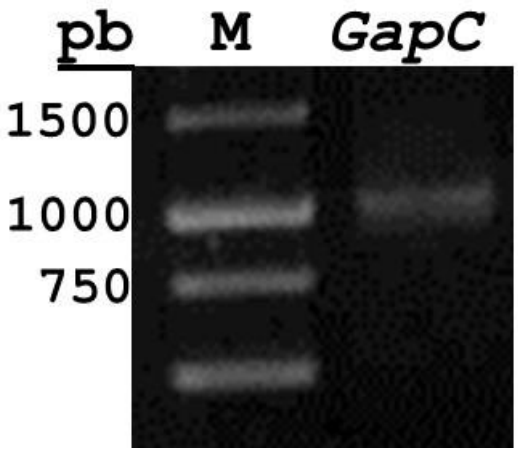
Polymerase Chain Reaction (PCR), Cloning and Sequencing. The total DNA molecule is then used as a template to amplify the *GapC* gene using the primer

pair of GPDX7F / GPDX9R. The *annealing* temperature used is 52.3 ° C. Components and PCR programs follow Roslim et al. (2018). The target gene PCR product is then inserted into a pTA2 vector (Toyobo) and then transformed into the bacterial cell *Escherichia coli* Zymo 5α (Zymo Research). Transformed colonies were amplified using T3 and T7 promoter primers. Plasmids from selected colonies were then isolated using ZR Plasmids MiniPrep (Zymo Research) and sequenced using T3 and T7 promoter primers.

Data Analysis. The DNA sequence data was analyzed by bioinformatics using the BLASTn program. The GapC sequence of 10 accessions that appeared in the BLASTn analysis was downloaded to create a dendrogram using MEGA6 software with Neighbor Joining Tree, Kimura-2-Parameter model and 1000 bootstraps.

RESULTS AND DISCUSSION

PCR products from the *GapC* gene in the *Elaeocarpus floribundus* plant have been obtained with a size of around 1000 bp (Picture 1). These results are not much different from those found in *Hibiscus tiliaceus* plants. Amplification of the *GapC* gene in *H. tiliaceus* plants produced a band of about 950 bp (Wu et al., 2007).



Picture 1. DNA band profile of the *Elaeocarpus floribundus GapC* gene in 1.2% agarosa gel. pb = base pairs; M = 1 kb of DNA Ladder (Thermo Scientific); GapC = DNA band from the *GapC* gene.

The *GapC* gene PCR product in this study appeared thin when electrophoresed. The thin *GapC* gene band obtained in this study shows the amount of amplification products is small. According to Olsen & Schaal (1999) and Wu et al. (2007) the *GapC* gene in plants is included in the nucleus with a *low copy number*. Therefore, cloning was carried out on the plasmid vector so that sequencing could be carried out properly and the DNA sequences obtained were accurate. The sequencing results of the *E. floribundus GapC*

gene were 933 bp. The sequence has been registered in GenBank database with registration number MK942072 (Picture 2).

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> MK942072| Elaeocarpus floribundus glyceraldehyde 3-
phosphate dehydrogenase (GapC) gene, partial sequence
TGATAGATTTGGAATTGTTGAGGGTCTCATGACCACTGTCCATTCCATTACTGGTGAGTA
TACATATTATGATGTATTTTAACTAGTTGCAACAGATGTAGATTCTTGATTGGCTTATTT
TTCTGTGTCATTCAGCCACCCAAAAGACTGTTGATGGCCCATCAATGAAGGACTGGAGAG
GTGGTAGAGCTGCTTCCTTCAACATCATTCCAAGCAGTACTGGCGCTGCTAAGGTATACG
ATGATTGTATTTATCAATTGACATTCAACTATCTTATATTGCATTATATCCTTTCTTTTG
CATTTGCAATTGTGGTTGTAAAGAATCCCTTATCCTCTGTTGCACAAGAGAATCCATAGT
TTGCGTGACTACAATGCTTGTGTTCTATTTGTACTTGTGTAGGCTGTTGGGAAAGTGCTG
CCAGCACTGAATGGGAAATTGACTGGAATGGCTTTCCGTGTTCCTACTGTTGATGTCTCA
GTGGTTGACCTCACTGTAAGACTCGAGAAGAATGCCTCTTATGCGGACATCAAAGCTGCT
ATCAAGTAAGGAACAGTGATGTTTCCTTTCATCTTCATTTTTGTAAATTGATAATTGTAT
TGCTCAAGTTATTAGTAGATGCAAACCAGATGCGTTCAGTTTGATCTATTAAATAGTGAC
TTTTCCAATCTTATTCCTTGATCACTCACTCTTTAACCCCGTTCTAATTCTTCTCCAACT
TTCTTAGGGAGGAATCTGAGGGCAACCTGAAGGGAATCCTTGGCTACACTGAGGATGATG
TGGTGTCAACTGACTTCGTTGGTGATAGCAGGTGAAGAGGTTTTGCATGAGCATATTTAC
ATGAGATCACTTTCTCTTTGGGCATAGTGATAAATATTTATAATTGCATCTTGGCAGGTC
GAGCATTTTTGATGCCAAGGCTGGAATTGCTTA
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Picture 2. DNA sequence of the *GapC* gene from *Elaeocarpus floribundus*.

Using the same primer, Olsen & Schaal (1999) obtained the *GapC* gene in cassava (*Manihot esculenta*) with a size of 962 bp. Wu et al. (2007) have also obtained the *GapC* gene in *H. tiliaceus* plants with a size of 935 bp.

The *E. floribundus GapC* gene sequence obtained in this study is predicted to consist of 4 introns and 5 exons. These results are consistent with those obtained in cassava plants (Olsen & Schaal, 1999). The position of the exons in the *E. floribundus GapC* gene is in nucleotides 1-53, 136-233, 403-545, 728-811, and 898-933. The total exon is estimated to be around 409 bp and encodes 137 amino acid deductions.

The results of the BLASTn *GapC E. floribundus* sequence show that the *E. floribundus GapC* sequence has a high similarity, which is 89.84% -90.16% with several species from the Cunoniaceae family (Table 1).

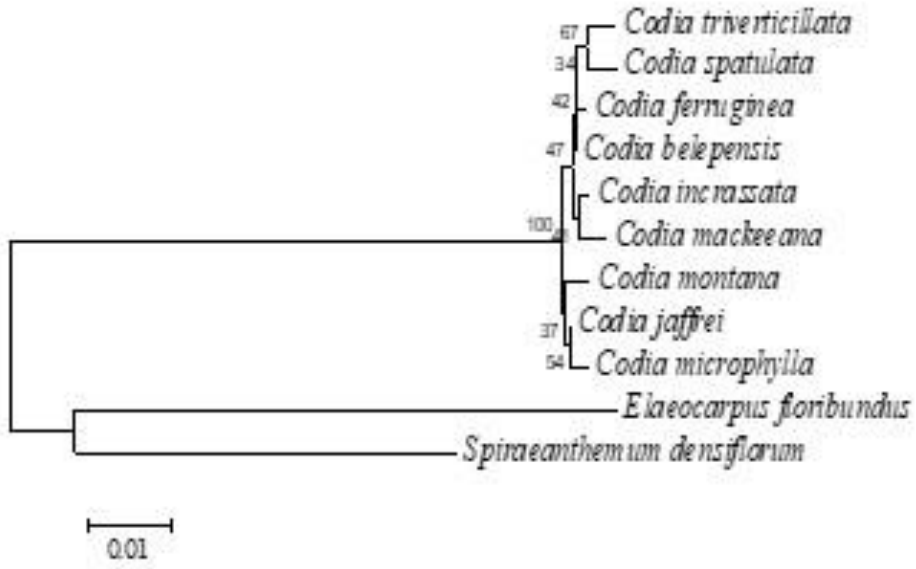
The same gene from several different species will have many similarities. High similarity shows that the gene has the same role in cells (Claverie & Notredame, 2007).

In this study, the *E. floribundus GapC* gene sequence has a high similarity with several plant species with different families because the *GapC* gene in all plants plays a role in the process of glycolysis.

Table 1. BLASTn analysis results on the *Elaeocarpus floribundus GapC* gene sequence.

Species	Query Cover (%)	Identity (%)	Family
S. densiflorum	90	89.84	Cunoniaceae
C. triverticillata	85	90.16	Cunoniaceae
C. spatulata	85	90.16	Cunoniaceae
C. montana	85	90.16	Cunoniaceae
C. microphylla	85	90.16	Cunoniaceae
C. mackeeana	85	90.16	Cunoniaceae
C. jaffrei	85	90.16	Cunoniaceae
C. incrassata	85	90.16	Cunoniaceae
C. ferruginea	85	90.16	Cunoniaceae
C. belepensis	85	90.16	Cunoniaceae

The dendrogram shows that the *E. floribundus GapC* sequence forms a group with *Spiraeanthemum densiflorum* and is separated from species of *Codia* genus (Figure 3). This result shows that the *E. floribundus GapC* sequence has more similarities with the *S. densiflorum GapC* sequence.



Picture 3. Dendrogram are based on the *GapC* gene sequence.

The *GapC* gene obtained in this study further needs to be validated - one of which is the quantitative *real time PCR* (qRT-PCR) technique - to determine whether this gene can be used as an internal control in *E. floribundus* plants. The *GAPDH* gene has been used as an internal control in several plants such as dates (*Phoenix dactylifera*) (Patankar et al., 2016), *pigeonpea* (*Cajanus cajan*) (Sinha et al., 2015), citrus (Mafra et al., 2012) and olive (*Olea europaea*) (Ray & Johnson, 2014).

Aside from being as an internal control, the *E. floribundus GapC* gene can also function as a marker of DNA contamination in cDNA molecules isolated when gene expression studies are carried out, because the *GapC*

gene obtained in this study contains introns and exons. The trick is to amplify the *GapC* gene using a template in the form of DNA and cDNA separately. The PCR products with DNA as a template will be 933 bp while cDNA as a template will be 409 bp.

CONCLUSION

The partial *GapC* gene sequences obtained in this study were 933 bp in size, consisting of 4 introns and 5 exons, and encoded 137 deduction amino acids. BLASTn analysis shows that the sequences obtained in this study have similarities of 89.84% -90.16% and the query cover of 85% -90% with species from the family Cunoniaceae, namely species of the genus Spiraeanthemum and *Codia*. Sequences of the partial *GapC* gene of *E. floribundus* are more similar to sequences in *Spiraeanthemum* compared to species of the genus *Codia*. The partial *GapC* gene sequence of *E. floribundus* obtained in this study was the first to be reported from the Elaeocarpaceae family.

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REFERENCES

Claverie, J.M. & Noterdame, C. (2007). Bioinformatics for Dummies, 2nd Editions. Indianapolis, Indiana: Wiley Publishing, Inc.

Elvyra, R. & Yus, Y. (2012). Ikan Lais dan Sungai Paparan Banjir di Provinsi Riau. Pekanbaru: UR Press Pekanbaru.

Ezin V., Vodounon, C.A., de la Peña, R., Ahanchede, A. & Handa, A.K. 2012. Gene Expression and Phenotypic Characterization of Flooding Tolerance in Tomato. *Journal of Evolutionary Biology Research* 4(3): 59-65.

Kozera, B. & Rapacz, M. (2013). Reference Genes in Real-time PCR. *J Appl Genetics* 54:391–406.

Mafra, V., Kubo, K.S., Alves-Ferreira, M., Ribeiro-Alves, M., Stuart, R.M., Boava, L.P., Rodrigues, C.M. & Machado, M.A. (2012) Reference Genes for Accurate Transcript Normalization in Citrus Genotypes under Different Experimental Conditions. *PLoS ONE* 7(2): e31263. doi:10.1371/journal.pone.0031263

Olsen, K.M. & Schaal, B.A. (1999). Evidence on the Origin of Cassava: Phylogeography of *Manihot sculenta*. *Proc. Natl. Acad. Sci. USA* 96: 5586-5591.

Patankar, H.V., Assaha D.V.M., Al-Yahyai, R., Sunkar, R. & Yaish, M.W. (2016). Identification of Reference Genes for Quantitative Real-Time PCR in Date Palm (*Phoenix dactylifera* L.) Subjected to Drought and Salinity. *PLoS ONE* 11(11): e0166216. doi:10.1371/journal.pone. 0166216.

Ray, D.L. & Jhonson, J.C. (2014). Validation of reference genes for gene expression analysis in olive (Olea europaea) mesocarp tissue by quantitative real-time RT-PCR. *BMC Research Notes*. 7:304.

http://www.biomedcentral.com/1756-0500/7/304

Roslim, D.I., Azrial, Herman & Lestari, W. (2018). The *GAPDH* Partial Gene of Durik-Durik (*Syzygium* sp.) from Riau Province of Indonesia. *IOP Conf. Series: Journal of Physics: Conf. Series*. 1116: 052055.

Roslim, D.I., Khumairoh, S. & Herman. (2016). Confirmation of Tuntun Angin (*Elaeocarpus floribundus*) Taxonomic Status Using matK and ITS Sequences. *Biosaintifika: J. Biol. Biol. Edu*. 8(3): 392-399.

Sinha, P., Saxena, R.K., Singh, V.K., Krishnamurthy, L. & Varshney, R.K. (2015). Selection and validation of housekeeping genes as reference for gene expression studies in pigeonpea (Cajanus cajan) under heat and salt stress conditions. *Front. Plant Sci*. 6:1071. doi: 10.3389/fpls.2015.01071.

Strand, A.E., Leebens-Mack, J. & Milligan, B.G. (1997). Nuclear DNA-based markers for plant evolutionary biology. *Mol. Ecol.* 6, 113-118.

Wang, M-L., Li, Q-H., Xin, H-H., Chen, X., Zhu, X-J., Li X-H. (2017). Reliable Reference Genes for Normalization of Gene Expression Data in Tea Plants (*Camellia sinensis*) Exposed to Metal Stresses. *PLoS ONE* 12(4): e0175863. https://doi.org/10.1371/journal.pone.0175863.

Wu, L., Tang, T., Zhou, R. & Shi, S. (2007). PCR-mediated Recombination of The Amplification Products of the *Hibiscus tiliaceus* Cytosolic Glyceraldehyde-3-phosphate Dehydrogenase Gene. *Journal of Biochemistry and Molecular Biology* 40(2): 172-179.

Zeng L, Deng R, Guo Z, Yang S, Deng X. 2016. Genome-wide identification and characterization of Glyceraldehyde-3phosphate dehydrogenase genes family in wheat (Triticum aestivum). *BMC Genomics* 17: 240.